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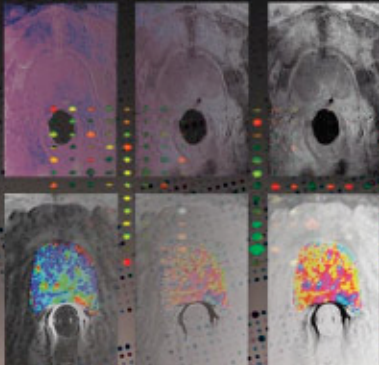
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Co-localizing BCE-1MRI with Molecular Profiles
in Prostate Cancer

3D Image Analysis of Fluorescent Drug Binding

An Imaging Agent for Rubio-Johnson Syndrome



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An Interventional MRI Technique for the Molecular Characterization of Heterogeneous Intra-Prostatic Dynamic Contrast Enhancement

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Abstract

The biological characterization of an individual patient's tumor by non-invasive imaging will have an important role in cancer care and clinical research if the molecular processes that underlie the image data are known. Spatial heterogeneity in the dynamics of MRI contrast enhancement (DCE-MRI) is hypothesized to reflect variations in tumor angiogenesis. Here we demonstrate the feasibility of precisely co-localizing DCE-MRI data with the genomic and proteomic profiles of underlying biopsy tissue using a novel MRI-guided biopsy technique in a patient with prostate cancer.

Abbreviations:

DCE-MRI – Dynamic Contrast Enhanced Magnetic Resonance Imaging

GKM – General Kinetic Model

MR – Magnetic Resonance

MRI – Magnetic Resonance Imaging

ROI – Region of Interest

Introduction

Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) provides a visual representation of both the anatomy and microvascular biology of cancer by measuring temporal changes in MR signal intensity associated with the intravascular injection of a contrast agent. (1) Spatial heterogeneity in the kinetics of contrast transit is thought to reflect variations in tissue perfusion and microvascular permeability. (2) Angiogenic microvessels, important for the growth and survival of cancer cells, are characterized in part by larger endothelial cell gaps resulting in greater permeability to small molecules. (3) Kinetic analysis of DCE-MRI is thus hypothesized to create an image reflecting the underlying malignant vasculature of an individual patient's tumor. There is mounting incentive to incorporate imaging surrogates, such as DCE-MRI, for patient selection and early measures of response in clinical trials of molecularly targeted anti-angiogenic therapies. (4) Imaging has the potential to provide more complete information on a tumor's microvascular biology, in contrast to information obtained from a biopsy, which may be subject to sampling error. In addition, imaging is non-invasive and spares the potential morbidities of biopsy, lending itself to serial measurements through a course of therapy.

However, data elucidating the molecular processes that underlie DCE-MRI and establishing its validity as a surrogate are lacking. Notable intraprostatic (5) and intratumoral (6) heterogeneity mandates millimeter co-localization accuracy between tissue samples and their corresponding image pixels. When prostate MR imaging and tissue acquisition procedures are performed in different settings and at different times, clinical co-registration is fraught with error. To address this key issue, we developed a technique for

MRI-guided needle biopsy of the prostate to be performed concurrently with a diagnostic MRI procedure inside a cylindrical 1.5T MRI scanner.

Methods

A patient with Stage I, intermediate-risk localized prostate cancer provided informed consent for enrollment on this IRB approved study. For the integrated procedure, the patient is positioned prone and a custom-designed interventional endorectal imaging coil is inserted and secured to the scanner table. A needle guide inside the stationary imaging coil contains MR tracking microcoils allowing for spatial registration of the device (R.C.S. et al, manuscript submitted, personal communication) (7). A continuous series of DCE-MR images of the prostate (3D spoiled GRE, scan time 5.1s, **Fig. 1A**) are acquired before and during the injection of intravenous contrast (gadolinium chelate, 0.2mmol/kg, 3cc/s). The needle guide is translated and rotated within the endorectal coil until its trajectory, computed from the tracking coils, coincides with a biopsy target location defined on the diagnostic images. A 14G core biopsy needle is then inserted, its location is verified by MRI, and tissue is collected. (**Fig. 1B and C**) This can be repeated for additional biopsy target sites within the prostate gland. The overall imaging and procedure time is approximately 90 minutes depending on the number of biopsies.

To analyze DCE-MRI data, a T1 map of the prostatic anatomy is first generated (8) to estimate the concentration of gadolinium chelate for a given signal intensity. Pixel data are submitted to a general kinetic model (GKM) fitting routine (9), which corrects the data for arterial input kinetics (measured over the external iliac artery) and implements a curve-

fitting solution to a GKM convolution integral. In this fashion, regions of interest (ROIs) encompassing those MR image pixels that correspond to the biopsy locations can be defined, and their corresponding time-intensity profiles and summary kinetic parameters computed. **(Fig. IE)** The transfer constant K^{trans} (corresponding to the magnitude of the enhancement curve, unit min^{-1}) and the rate constant k^{ep} (describing the rate of clearance, unit min^{-1}) are thought to reflect differences in tissue perfusion and microvascular permeability, respectively. The kinetic parameters are derived based on the following equation:

$$C_T(t) = K^{\text{PS}} \int C_P(\theta) e^{-k(t-\theta)} d\theta + f_{\text{PV}} C_P(t)$$

Where $C_T(t)$ = concentration of gadopentate in tumor tissue at any time t , $K^{\text{PS}} = K_{\text{TRANS}}$ = endothelial transfer coefficient, C_P = concentration of gadopentate in the plasma space of the tumor tissue (assumed equal to that in the central venous blood plasma, i.e. input function), k = rate constant of reflux from interstitial water back to plasma, and f_{PV} = fractional plasma volume of the tumor tissue.

To characterize the biological processes underlying the image data, needle biopsy specimens can be subjected to comprehensive histopathological, genomic, and proteomic analysis. Such analysis is chiefly enabled by microarray technology, which is distinguished by its comprehensive analytic capabilities using low sample volumes. In this case example, mRNA was isolated and amplified from snap frozen cores (10). The amplified mRNA was co-hybridized to a cDNA microarray with a reference standard. In turn, whole cell protein lysates from ethanol-fixed and paraffin-embedded tissue sections of

twin cores obtained at the same biopsy sites were analyzed using reverse phase protein arrays. (Ref 11 for detailed methods)

Early Results

We focused our initial analysis on signaling pathways known to be associated with angiogenesis. This case example shows differing levels of protein and gene expression at distinctive sites of contrast enhancement kinetics on DCE-MRI. (**Fig. 1I**) The level of hypoxia inducible factor (HIF-1 α) mRNA and protein was lower at the site of higher contrast enhancement, while a number of other genes involved in angiogenesis signaling were upregulated. Some discordance observed at a single time point between protein and corresponding mRNA, for example AKT levels, supports the need for a comprehensive and serial analysis to evaluate mRNA/protein kinetics.

Conclusion

Our results show that the technical challenge of integrating needle-based prostate interventions with diagnostic MRI in a cylindrical clinical scanner can be overcome. Image subsites of interest can be precisely sampled, providing a research platform well suited to MRI and tissue correlation. The molecular profile prostate tissues underlying DCE-MRI will now be acquired in a larger series of patients in order to characterize the molecular biology of MR contrast enhancement. As we gain knowledge in the molecular biology underlying cancer and DCE-MRI, a more valid interpretation of an individual patients' tumor biology will ensue.

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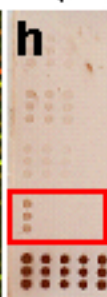
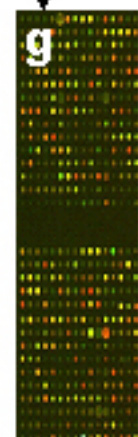
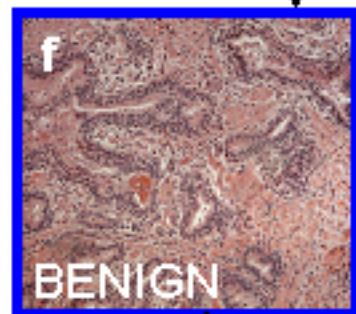
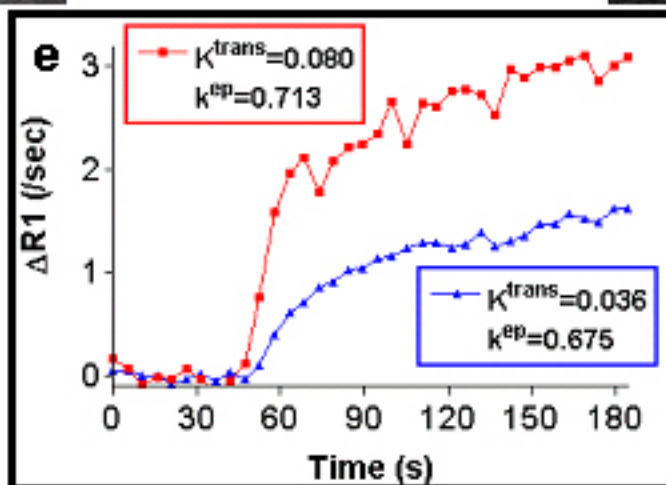
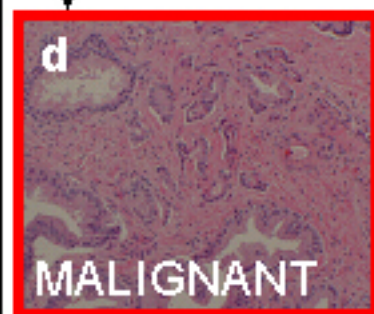
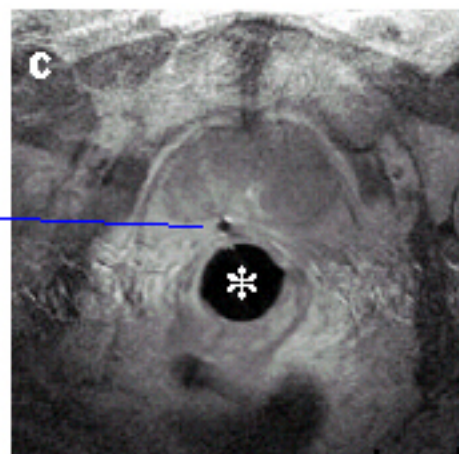
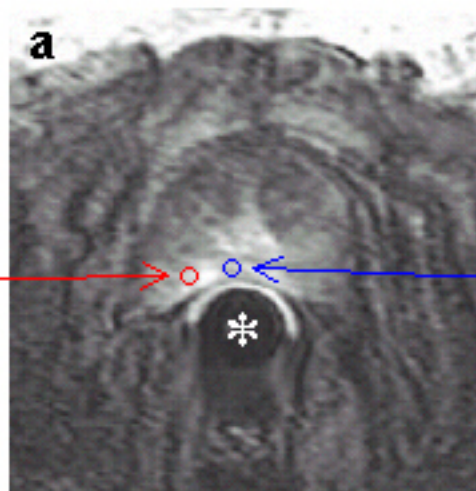
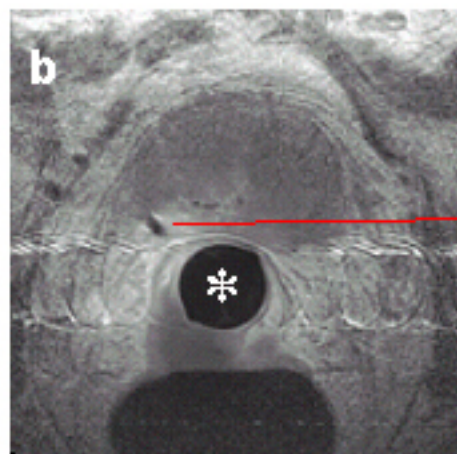
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Figure Legends

Figure I: Prostate interventional MRI for the correlation of molecular biology and DCE-MRI. The stationary interventional endorectal coil (*) is used for both diagnostic and interventional MR imaging. **(a)** DCE-MRI at 120s shows a small area of increased signal intensity in the left peripheral zone of the prostate. ROIs (red and blue) corresponding to the subsequent needle biopsy voids **(b,c)** are defined for image analysis. **(e)** Time-intensity curves (corrected for T1 heterogeneity) from each ROI are fit to a GKM convolution integral using an arterial input function measured from the external iliac artery. The transfer constant K^{trans} (corresponding to the magnitude of the enhancement curve, unit min^{-1}) and the rate constant k^{ep} (describing the rate of clearance, unit min^{-1}) are thought to reflect differences in the perfusion and microvascular permeability underlying each ROI, respectively.² H&E staining shows adenocarcinoma **(d)** corresponding to higher K^{trans} and k^{ep} than benign tissue **(f)**. cDNA microarray **(g,k)** and reverse phase protein array **(h,j)**-array probed with STAT3 antibody shown) (11) results show differing trends of protein and gene expression levels **(i)** from benign (blue ROI) to malignant (red ROI) tissue obtained at distinctive sites of contrast enhancement kinetics on DCE-MRI.



i	Array	Pathway	↑		↓	
cDNA Microarray	Hypoxia		NOS3, VHL		HIF1A	
	PDGF		MAPK8, STAT3, PDGFRA		FOS, JUN, MAP2K1&4	
	AKT		AKT1		EIF2S1&3	
Protein Array	Hypoxia		-		HIF1a, eNOS	
	PDGF		-		PDGFβ, pPDGFβ, STAT3	
	AKT		-		AKT, pAKT, EIF4G	

